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Flow Injection Chemiluminescence Determination of Paraquat using Luminol and Ag(III) Complex

FEI LIU[1], HONGMEI SHI[1], XIANGDONG XU[1], WEIJUN KANG[1] and ZHANHUA LI[2,*]

[1]School of Public Health, Hebei Medical University, East Zhongshan Road 361, Shijiazhuang, Hebei Province 050017, P.R. China
[2]Basic Medical College, Hebei Medical University, East Zhongshan Road 361, Shijiazhuang, Hebei Province 050017, P.R. China

*Corresponding author: Fax: +86 311 86265754; E-mail: lizhanhua99@126.com

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A novel flow injection chemiluminescence system is described for the determination of paraquat. The method is based on the fact that paraquat can enhanced the chemiluminescence intensities of Ag(III)-luminol in alkaline solution. The optimum conditions for the chemiluminescence emission are evaluated. Paraquat can be determined over the concentration range of 5.0×10^{-6}-1.0×10^{-3} M with a correlation coefficient of 0.999 and a detection limit of 2.0×10^{-7} M (S/N = 3). The relative standard deviation for 11 repetitive determination of 2.0×10^{-4} M paraquat is 1.01 %. The proposed method offers the potential advantages of high sensitivity, simplicity and rapidity for paraquat determination and the utility of this method is demonstrated by detecting paraquat in pesticide and vegetable samples.

Key Words: Ag(III)-Luminol system, Chemiluminescence, Paraquat determination.

INTRODUCTION

Paraquat dichloride (methylviologen; PQ) is a non-selective herbicide that has been used widely throughout the world. Although it has a proven safety record when appropriately applied to eliminate weeds, paraquat is known to causing toxic effects and produce free radicals in animals and man[1,2]. Severe paraquat poisoning is characterized by multiple-organ failure, involving lungs, kidney, liver, myocardium and adrenal[3]. Owing to its widespread use and toxic effect, considerable attention has been paid to develop sensitive and rapid analytical techniques for the determination of paraquat.

Conventional methods including gas chromatography and high performance liquid chromatography are available to monitor the paraquat in literature, but are limited by the high cost, the time-consuming and low sensitivity[4,5]. LC–MS is a promising technique but requires expensive instrumentation.

Chemiluminescence (CL) is extensively studied as an attractive detection means for trace analysis in biotechnology, medical diagnostics and environment chemistry due to its simplicity, rapidity and high sensitivity. Luminol is one of the earliest and most common chemiluminescence reagents used in chemiluminescence reaction. It can be oxidized by some strong oxidants such as H_2O_2, $K_3Fe(CN)_6$, $KMnO_4$ and BrO^-. Although luminol system has been used for various applications, the chemiluminescence reaction is scarcely applied to the detection of pesticides. Luminol-H_2O_2 system was couple with HPLC and used for the selective detection of organophosphorus insecticides in vegetable[6], a flow injection chemiluminescence method has been proposed to determine some dithiocarbamate fungicides, such as ziram, mancozeb[7], based on the oxidation of luminol in alkaline medium, the detection limits of ziram and mancozeb were 6.6×10^{-9} and 3.8×10^{-10} M.

As a new oxidant in chemiluminescence reaction, Ag(III) complex, $[Ag(HIO_6)_2]^{5-}$, coupled with luminol was applied successfully for determinations of cortisol in human blood sera[8]. The Ag(III) complex, having a square-planner geometry around the metal center[9-12], is fairly stable in alkaline media[13]. The structure of the Ag(III) complex[11] is illustrated in Fig. 1.

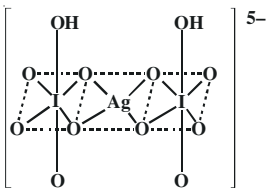

Fig. 1. Structure of the Ag(III) complex

In this work, the effect of paraquat on the luminol-Ag(III) system was investigated. It was found that paraquat could dramatically enhance chemiluminescence intensities in alkaline so-

lution. Based on the enhancement effect of paraquat, a new, rapid, simple, sensitive and inexpensive method was proposed for the determination of paraquat in flow injection analyses (FIA). The detection limit of this method was as low as 2.0×10^{-7} M for paraquat and the relative standard deviation was 1.01 %. Under the optimized conditions, the proposed flow injection chemiluminescence system was applied for the determination of paraquat in pesticide and vegetal samples.

EXPERIMENTAL

Analytical standard of parequat was purchased from Institute for the Control of Pharmaceutical Products (Beijing, China). A stock solution of 1.0×10^{-2} M paraquat was prepared by dissolving accurately weighted amounts in water and stored in darkness at 4 ºC. Working standard solutions were freshly prepared by dilution concentration with water. A 0.020 M stock of luminol, obtained from Merck (Merck, Darmstadt, Germany), was prepared by dissolving 0.8860 g of luminol in 7.00 mL 1.00 M NaOH and then diluted with water to 250 mL. $AgNO_3$, KIO_4, $K_2S_2O_8$, KOH and CTAB were obtained from Beijing Chemical Reagent Company (Beijing) or from Tianjin Chemical Reagent Company (Tianjin, China). All the chemicals used were of analytical reagent grade. Double-distilled water (referred to pure water thereafter) was used as carrier flow and for preparation of solutions.

Bis(hydrogen periodato)argentate(III) complex anion, $[Ag(HIO_6)_2]^{5-}$, was synthesized according to the procedure described previously[13]. Electronic spectra of aqueous solutions of the Ag(III) complex gave rise to two absorption bands centering at 362 and 253 nm, which were in excellent agreement with those reported earlier[9]. Stock solutions of the Ag(III) complex, prepared from the solid-state compound obtained[13], was fresh and used daily. Their concentrations were determined spectrophotometrically at 362 nm by using of the molar absorptivity of $\varepsilon = 1.26 \times 10^4$ M^{-1} cm^{-1} [9].

Sample handling and extraction procedure

Pesticide sample: The sample was prepared by appropriate dilution of pesticide to the concentration within the working range.

Vegetable sample: Spinach samples were purchased from a local market. A pre-treatment step consisting of solid phase extraction (SPE) was conducted, based on a previously reported[14], in order to reach sensitivity levels below the legal maximum concentration permitted. Several pieces of spinach were chopped and homogenized. The above vegetable sample was soaked using methanol solution (pH = 4) for 12 h. After 10 min sonication, the mixture was filtered by filter paper and collected. 10 % NaOH was added to adjust pH to 7-9. The paraquat in the obtained solution was enriched according to the following steps, the eluate was injected directly for detection. (1) Condition: 5 mL methanol, 5 mL distilled water, 5 mL cetrimide, 10 mL sodium hexanesulfonate in turn. (2) Load: sample solution was applied to the cartridges. (3) Wash: 5 mL water. (4) Elute: 5 mL phosphoric acid added with diethylamine.

Fig. 2 shows the schematic diagram of the flow injection chemiluminiescence (FI-CL) system (Xi'fan Remex Electronic Science-tech, Xi'fan, China) used for the determination of paraquat. There were two peristaltic pumps (working at a constant flow rate: 40 rpm): one was used to propelled the flow stream of Ag(III) and luminol, the other was used to deliver the sample solution and carrier flow using PTFE tubing (0.8 mm i.d.). The flow cell was a 10-cm-long spiral glass tube and placed close to the window of the photomultiplier (PMT). A sample solution was injected by a six-way valve into the carrier stream and then merged with the mixture stream of Ag(III) and luminol solutions just reaching a flow cell previously to make an enhanced chemiluminescence signal. The signal intensity was immediately recorded by an analyzer. The pumps and the six-way injection valve with a sample loop were automatically operated by a computer equipped operation system of MPI-B flow injection analysis.

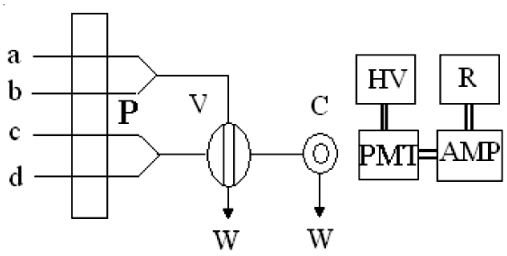

Fig. 2. Schematic diagram of flow injection chemiluminescence analysis system. P: peristaltic pump; V: sample inlet valve; C: flow cell; PMT: photomultiplier tube; AMP: amplifier; HV: high voltage; R: recorder; W: waste; (a) luminol solution; (b) Ag(III) solution; (c) carrier flow; (d) sample solution

Chemiluminescence signal measurements: The Ag(III) and luminol solutions were merged through a three-way pipe and then propelled into the flow line to make a basic signal, as shown in Fig. 2. A chemiluminescence reaction occurred and the signal was recorded to make a baseline. Sample solutions were injected from sample valve and passed to the flow cell by carrier flow, when the baseline was stable. The analyte (paraquat) then mixed and reacted with the reagent stream of luminol and Ag(III) in the chemiluminescence flow cell which had positioned in front of the detector window. The chemiluminescence signal intensity of luminol-Ag(III)-paraquat system was recorded using a computer, equipped with a data acquisition interface. Calibration graphs were constructed by plotting the intensity (peak height) of chemiluminescence signal *versus* the concentrations of paraquat.

RESULTS AND DISCUSSION

Optimization of the method

Carrier flow: A thorough and sequential optimization of the method was carried out in several steps in order to ascertain the best condition for determination. In order to check the influence of carrier flow, phosphate buffers at pH 6.86, 9.18 and 10.0, acidic aqueous solution at pH 4.0, basic aqueous solution at pH 11, 12 and 13 and simply pure water were tested. Thus, pure water was selected as carrier flow to obtain a stable and smooth baseline.

Effect of flow rate: The influence of the carrier flow rate on the chemiluminescence signal intensity of luminol-Ag(III) system was investigated for the optimization of parameters. The chemiluminescence intensity increased with increasing of flow rate, but if the rate was too fast a stable signal could

not be obtained. A flow rate of 2.5 mL min^{-1} was therefore selected for further studies.

Influence of luminol concentration: Investigation on the effect of luminol concentration from 1.0×10^{-8}-2.0×10^{-6} M on the chemiluminescence signal intensity was performed at the fixed concentrations of Ag(III) (2.5×10^{-5} M) and KOH (4.0×10^{-2} M), using water as a carrier. As shown in Fig. 3, a sharp increase was found with increasing concentration of luminol, but the base signal of chemiluminescence reaction was also increased. 2.0×10^{-7} M luminol was selected since it produced higher signal-to-noise (S/N) ratio than the other concentrations.

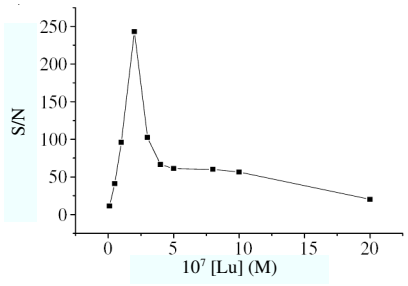

Fig. 3. Effect of luminol concentration on chemiluminescence intensity. Conditions: [Ag(III)] = 2.5×10^{-5} M (in 0.040 M KOH solution); [paraquat] = 2.0×10^{-4} M; luminol, in 0.005 M KOH solution; carrier flow, water

Effect of [Ag(III)] on chemiluminescence intensities: The concentration of Ag(III) could affect on chemiluminescence intensity of luminol-Ag(III)-paraquat system. It was found that higher concentration produced high chemiluminescence intensity, but further increase of Ag(III) complex caused a decline in chemiluminescence signal. 2.5×10^{-5} M Ag(III) was selected as the optimal concentration according to S/N ratio (Fig. 4).

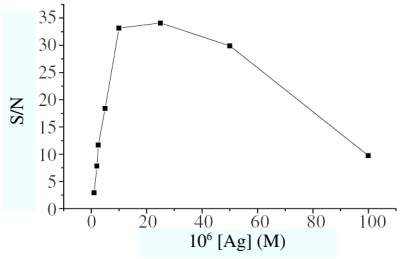

Fig. 4. Effect of Ag(III) concentration on chemiluminescence intensity. Conditions: [luminol] = 2.0×10^{-7} M (in 0.005 M KOH solution); [paraquat] = 2.0×10^{-4} M; Ag(III), in 0.040 M KOH solution; carrier flow, water

Effect of pH on chemiluminescence: The effect of pH on chemiluminescence reaction of luminol and Ag(III) were investigated. It has been reported that luminol reaction was under alkaline condition and the activity of luminol was extremely low in acidic solution[15]. The study showed that KOH concentration in luminol and Ag(III) was a significant factor for the determination of paraquat. Luminol and Ag(III) solution concentrations were studied 2.0×10^{-7} and 2.5×10^{-5} M, respectively. Considering the results obtained from chemiluminescence intensity (Fig. 5), the following optimum values were found for KOH concentration in luminol (5.0×10^{-4} M), in Ag(III) (1.0×10^{-1} M).

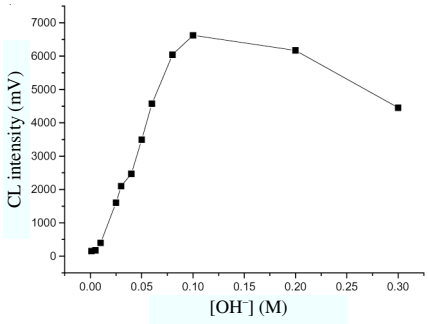

Fig. 5. Effect of $[OH^-]$ in Ag(III) solution on chemiluminescence intensity. Conditions: [luminol] = 2.0×10^{-7} M (in 0.005 M KOH solution); [paraquat] = 2.0×10^{-4} M; [Ag(III)] = 2.5×10^{-5} M; carrier flow, water

Interference studies: The effect of some inorganic ions and common co-existing compounds were tested as potent interferents. The chemiluminescence signals of standard paraquat solution were recorded and compared with that obtained for paraquat and interferents. A 5 % error criterion was adopted for the determination of paraquat in the concentration of 2.0×10^{-4} M. There was a slight interference from equal amounts of glucose and starch. The ratios of foreign substances tolerated in analysis were 100-fold for K^+, Na^+, Cl^-, SO_4^{2-}, NO^{3-}, PO_4^-, I^- and Br^-, 10-fold for Ag^+, Zn^{2+} and Ca^{2+}, 5-fold for Ba^{2+} and 2-fold for Fe^{3+}, Co^{2+}.

Calibration curve and performance characteristics: Under the optimal condition above described, the linearity for the determination of paraquat was investigated. The plot of the chemiluminescence intensities *versus* [paraquat] was linear in the range of 5.0×10^{-6}-1.0×10^{-3} M. A regression equation was obtained as: intensity = $375.60 + 2.95 \times 10^7$ [paraquat] (r = 0.999). The detection limit (S/N = 3) for the regression equation was 2.0×10^{-7} M and the relative standard deviation (RSD, n = 11) was 1.01 % for 2.0×10^{-4} M paraquat. Fig. 6 shows typical calibration traces recorded for paraquat using the proposed chemiluminescence system.

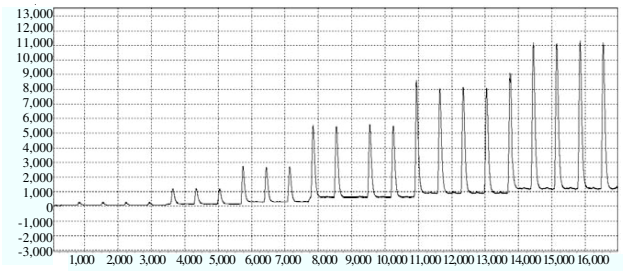

Fig. 6. Typical chemiluminescence signal of paraquat at different concentrations. [Luminol]: 2.0×10^{-7} M (0.005 M KOH); [Ag(III)]: 2.5×10^{-5} M (0.1 M KOH); carrier, water

Compared to other published methods, this new chemiluminescence system is highly sensitive and has a detection limit much lower than that by SPE-HPLC-UV(2.6×10^{-6} M)[14] and ELISA (7.8×10^{-6} M)[16].

Analytical applications

Analysis of pesticide sample: The diluted sample was injected and analyzed using the proposed method. It was showed that the concentration of paraquat in pesticide sample was lower than descried in introduction (7.8×10^{-1} M).

We also detected the samples using HPLC[14]. Chromatographic separation was performed on a Kromasil C18 column (5 μm, 250 mm × 4.6 mm i.d.). The mobile phase was phosphate buffer (0.18 mol/L) with sodium hexanesulfonate (18 mmol) and methyl cyanides (9:1) and detected at the wavelength of 256 nm. The results are in good agreement with those concentrations using chemiluminescence (Table-1).

TABLE-1
DETERMINATION OF PARAQUAT IN PESTICIDE

Sample	Nominal (10^{-1} M)	Proposed method (10^{-1} M)	HPLC method (10^{-1} M)
1	7.80	5.71	5.05
2	7.80	6.53	5.93

Analysis of Spinach samples: After the pro-treatment procedure as described under materials and methods, the sample was injected directly for the determination. No paraquat residues were detected from a sample by applying the proposed method. The internal standard was added before extraction and the absolute recovery are in the range of 85.4-109.2 % (Table-2).

TABLE-2
DETERMINATION OF PARAQUAT FROM VEGETABLE

Sample	Found (10^{-5} M)*	Added (10^{-5} M)	Recovered (10^{-5} M)*	Recovery (%)
1	0.00	2.00	1.71 ± 0.08	85.4
2	0.00	8.00	6.92 ± 0.03	87.3
3	0.00	20.00	21.81 ± 0.05	109.2

*Mean of three determination with standard deviation.

Conclusion

There is a great enhancement produced by paraquat on the chemiluminescence emission of luminol using Ag(III) as oxidant. A new chemiluminescence method is developed and validated for the determination of paraquat in pesticide and vegetable samples. The increasing public concern in recent years about possible health risk due to pesticide residues in the environment led to more stringent requirement on the limits of detection (LODs). Comparing with HPLC, GC and ELISA the proposed method is speed and simplicity of instrument. The chemiluminescence system based on the reaction of Ag(III) complex with luminol has more advantages than other luminescence methods, it is characterized by high sensitivity, low detection limit and the possibility of simple and quick analysis. Present technique is more suitable for the determination of paraquat at trace level. It also offers the possibility for the construction of low-cost online automatic analyzer for pesticide monitoring in the environment.

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